Use of the ambr®250 in combination with high-throughput design and analysis tools for rapid, scalable USP development

Authors:

Dr Fern Slingsby and Dr Simon Dewar

Upstream Process Development
FUJIFILM Diosynth Biotechnologies, Billingham, UK

Correspondence to:
Fern Slingsby (fern.slingsby@fujifilmdb.com)

ABSTRACT

There have been many recent advances in high throughput (HT) technologies for upstream development, enabling processes to be developed in a fraction of the time compared with conventional methods. However, when applying this technology to biotherapeutic drug development, the suitability of the systems for developing large scale manufacturing processes and meeting regulatory demands needs to be demonstrated and ensured. Inclusive approaches encapsulating platform expression systems and fermentation technologies, parallel bioreactor systems, high throughput analytics and sophisticated design and data handling tools can ensure these regulatory demands are met. The ambr®250 (24way) parallel bioreactor system (which enables multiple, simultaneous fermentations to be carried out under fully automated control) has the confirmed reproducibility and scalability that is required, and should be used in combination with HT analytics tools (e.g. the Cedex BioHT analyser and Caliper electrophoresis system) to enable rapid analysis of the considerable amount of samples generated. Experimental design and visual analytics software packages such as Design Expert® and JMP® enable this information to be easily visualised, interpreted and modelled to improve process knowledge. This combined with Design of Experiments (DoE) and Quality by Design (QbD) principles and platform fermentation technologies enables rapid progression from gene to optimised high titre fermentation processes, maintaining the high levels of process understanding required to meet regulatory requirements and with earlier process optimisation lowering regulatory risk. This approach has been applied successfully by FUJIFILM Diosynth Biotechnologies in combination with its proprietary pAVEway™ expression system and platform fermentation technology to obtain high titre, robust and well characterised fermentation processes suitable for cGMP manufacture of biotherapeutic drug products.

INTRODUCTION

The use of recombinant protein technologies for the production of biotherapeutic drug products poses numerous challenges. High levels of process understanding and robustness are required for cGMP (current Good Manufacturing Practice) manufacture, however the development time taken to obtain these must be kept to a minimum in order to reduce costs and meet constraints such as asset slots and clinical requirements. Regulatory demands (quite rightly) are high, and the time and cost implications of ensuring these are met can be significant. Optimised and well characterised fermentation processes are required, ensuring high quality product is produced at suitable titres to maintain profitability and a practical manufacturing strategy. However, combining these requirements and maintaining standards can demand considerable time and resource. There is therefore a clear need for tools and approaches which can reduce this demand whilst maintaining the high standards and levels of process understanding required to develop robust, well characterised upstream manufacturing processes. It is also desirable that these tools and approaches are scalable and so applicable to the manufacturing equipment and facilities for which the processes are being developed. This provides confidence that the results obtained and so the decisions made are relevant, otherwise the potential implications could be costly in terms of money, time and resources.

Recent advances in high throughput fermentation technologies can help to meet these needs. The ambr250 (24+) parallel bioreactor system enables multiple fermentations to be carried out under fully automated control. High throughput analysis tools, for instance the Caliper LabChip electrophoresis system or the Cedex BioHT analyser, enable rapid and reliable processing of the large amount of samples generated and, in combination with platform fermentation technologies

such as Fujifilm's pAVEway expression system, rapid development of well understood, high yield fermentation processes can be achieved. In addition, Quality by Design (QbD) principles, which are increasingly being asked of by regulators, can easily be combined with high throughput and platform technology earlier on in the process than was possible with old technology, providing process understanding at an earlier stage in development when impacts can be mitigated and alterations made with minimal cost.

THE ambr250 (24way) PARALLEL BIOREACTOR SYSTEM

The ambr250 (24way) parallel bioreactor system consists of 24 bioreactors, each with a maximum 250ml working volume under fully automated control (*Figure 1*), and can run up to 24 individual fermentation processes in parallel. The ambr250 system has continuous control and/or monitoring of parameters including pH, temperature, dissolved oxygen, off-gas analysis – in fact all the control and monitoring available for larger, conventional vessels. All sampling and additions can be automated, meaning the required operator time is kept to a minimum and disposable, pre-sterilised bioreactors virtually eliminate any risk of contamination. The system uses bioreactors that are a scaled-down version of conventional vessels, complete with Rushton impellers, sparger or headspace gassing, pH and DO probes and up to four feed lines, ensuring the system accurately reflects the large scale equipment for which the processes are being developed.

***Figure 1*: FUJIFILM Diosynth Biotechnology's ambr250 (24way) parallel bioreactor system**

USING THE ambr250 FOR MICROBIAL UPSTREAM DEVELOPMENT

The ambr250 system can be employed at several stages during the upstream development process (see *Figure 2*). Strain screening, for instance, can be carried out directly into fermenter vessels using this system. Strain screening is usually carried out in shake flasks and selections made based upon those strains showing the best productivity and product quality. However, shake flasks are often a poor approximation for bioreactors and allow for the screening of only a few parameters at best (e.g. temperature and inducer concentration). Parameters such as oxygen

transfer are poor compared with larger vessels and conditions such as pH are neither closely monitored nor tightly controlled. Therefore, selection is unlikely to favour the best strains and conditions appropriate for larger fermenter systems. Using the ambr250, however, not only enables the best strains to be selected for growth and expression in fermenter vessels, but also allows for fermentation process optimisation to be carried out in parallel to strain screening. This means high quality data and process understanding can be obtained much earlier on in the development process, as well as reducing the overall development time required for this stage.

During subsequent process optimisation stages, utilising the ambr250 means that multiple Design of Experiment (DoE) fermentations can be carried out simultaneously in a scalable, reproducible system. This ensures the maximum amount of statistically relevant data is collected by exploring more parameters in the maximum available 24 runs per cycle in ambr250 (compared to traditional approach in larger vessels) thus enabling an optimal design space to be determined with greater process understanding and increasing process robustness.

The ambr250 system can also be employed during late phase characterisation (LPC) studies. This is due to its proven scalability, ensuring relevant data is obtained whilst providing the high levels of process understanding and operating ranges required to satisfy regulatory requirements. This significantly reduces the time and cost taken to complete these investigations when compared with conventional, larger scale bioreactors.

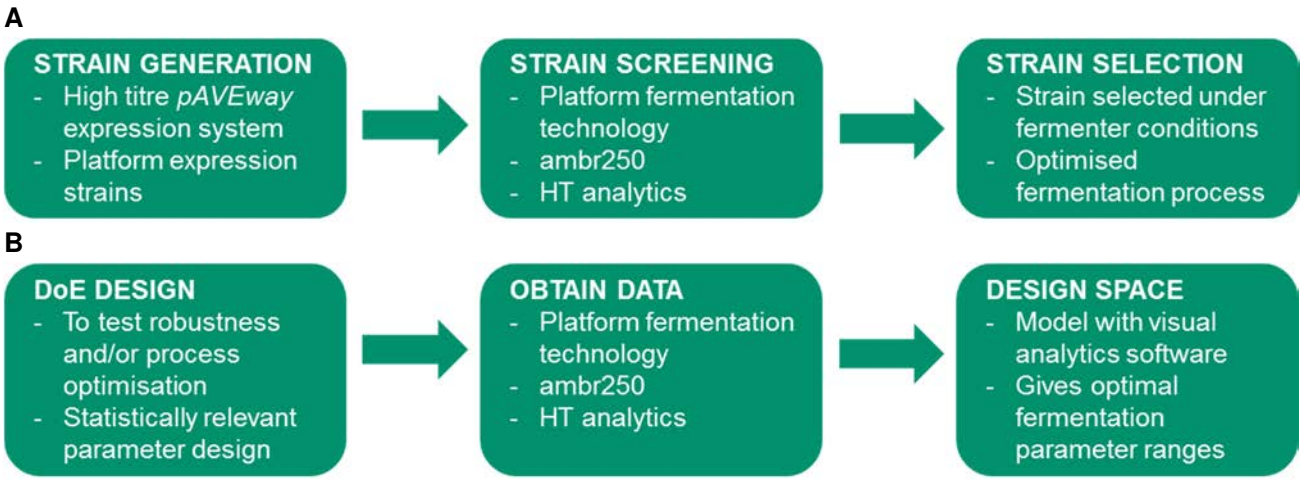


***Figure 2*: USP development process flow using FUJIFILM Diosynth Biotechnology's HT platform technology. A – Strain screening and fermentation process optimisation can be carried out in parallel. B – Further process optimisation utilises a DoE approach to determine the optimal design space.**

COMBINING QbD PRINCIPLES WITH THE ambr250

High throughput fermentation systems such as the ambr250 lend themselves perfectly for use in applying QbD principles to fermentation development, for instance when using a DoE approach to test robustness or for process optimisation or LPC studies (as discussed above). Designing and applying a statistically relevant DoE approach (for instance via software packages such as Design Expert and JMP) can assist in ensuring that the effects of changing each parameter on process operation and quality attributes are detected in the best possible manner and yet using the minimum number of fermentations. This means optimal operating ranges can be determined and

specific ranges set to ensure key quality attributes are maintained during the fermentation development process. However, this can still require a large number of fermentations and would not only be time consuming and expensive in larger vessels, but often needs additional fermentations beyond the minimum required for the number of parameters to be investigated. This is due to "blocking" designs, which are often necessary due to the practicalities of running multiple, parallel fermentations and takes into account any potential variation caused by carrying out fermentations on different days, in different fermenters and/or with different operators. The ability to run 24 fermentations in parallel using the ambr250 means that results can be obtained rapidly, possibly eliminating the need for blocking and still maintaining high levels of monitoring and process understanding – ensuring the minimum amount of experiments are carried out and enabling additional fermentations to be performed for the same resource when compared with conventional vessels.

HIGH THROUGHPUT SAMPLE ANALYSIS AND DATA PROCESSING

Of course there is always the potential for high throughput systems such as ambr250 to create bottlenecks downstream, for instance in sample processing and data analysis, simply due to the amount of data and samples generated over a much shorter time frame when compared with typical development programs. Conventional SDS-PAGE, for example, would not be practical for timely analysis of product expression in, say, four samples per 24 fermentations (or 96 per ambr250 run). High throughput analysis tools such as the Cedex BioHT analyser and Caliper electrophoresis systems can help, enabling multiple samples to be processed in a fraction of the time taken for conventional analysis techniques. In addition, the vast amount of high-quality data created by such systems needs to be processed in order to ensure that the most benefit is obtained from the impressive capabilities of the systems. After all, what's the point in collecting it if you're not going to use it? Utilising the power of software packages such as JMP can help process the information and provide visual analytics, statistical analysis and visual representation of the large quantities of information obtained, for instance by developing bespoke scripts for effective mining of this information and applying multivariate analysis to further define the design space at an earlier stage in the development timeline. Obtaining such information early on in the development process not only facilitates a more refined fermentation process, but a more in-depth understanding of manufacturing processes is advantageous from a regulatory point of view too, helping to prevent any unwanted surprises further on down the development pipeline.

CONFIRMING THE SUITABILITY OF THE ambr250 FOR UPSTREAM DEVELOPMENT

The suitability of the ambr250 for modelling and developing upstream fermentation processes comes from the reproducibility and scalability of the system. Such reproducibility was demonstrated when running an identical process in all bioreactors and comparing the on-line and off-line monitoring profiles. This is shown in *Figure 2*, where an *Escherichia coli* intracellular expression fermentation was carried out using an exponential feed following depletion and a 12 hour induction window following IPTG addition. Perfect overlaying of the traces for dissolved oxygen (DO), agitation, oxygen uptake rate (OUR) and carbon dioxide evolution rate (CER) was observed, and measurements on end of fermentation titre and dry cell weight were also comparable between the vessels.

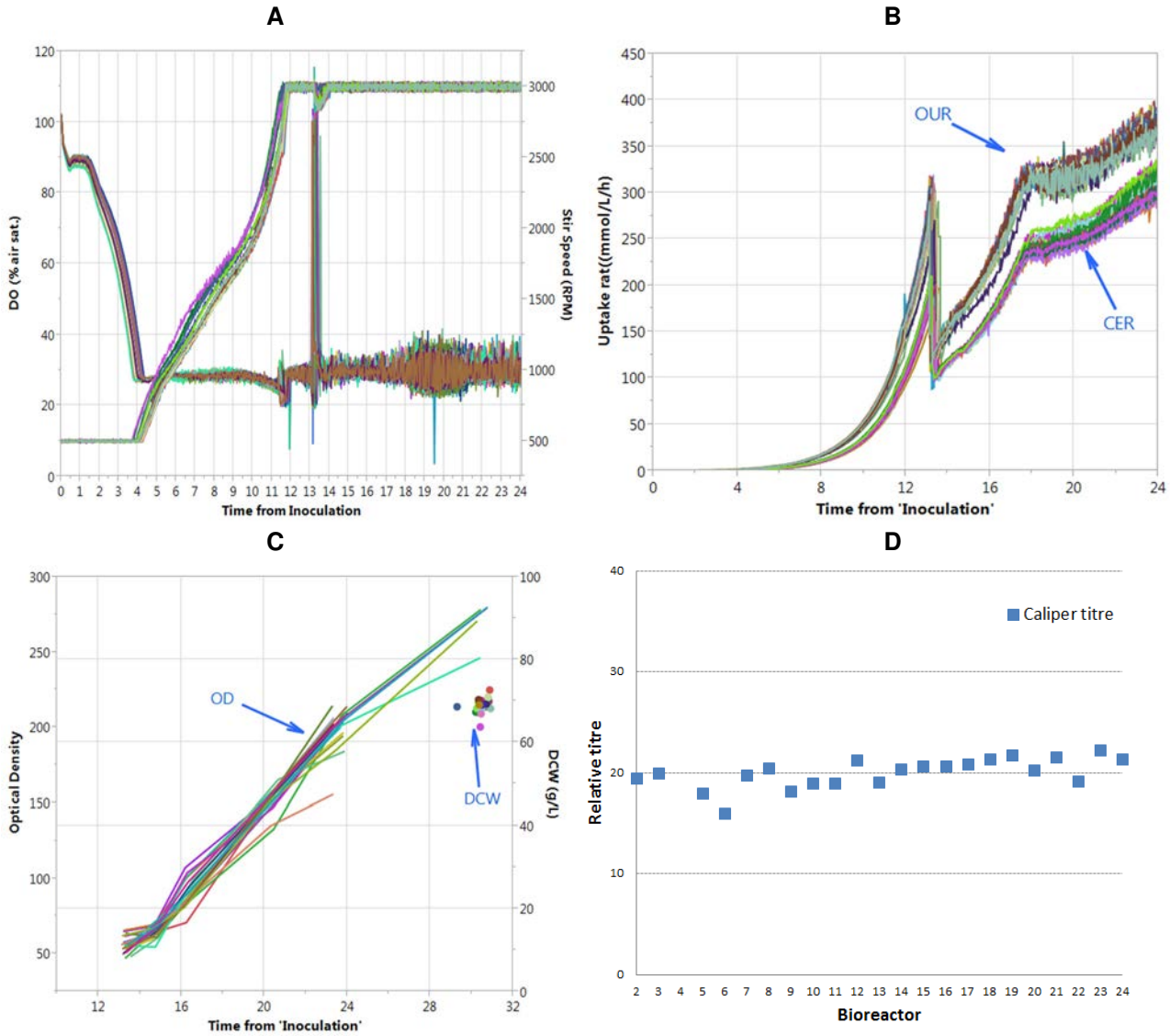
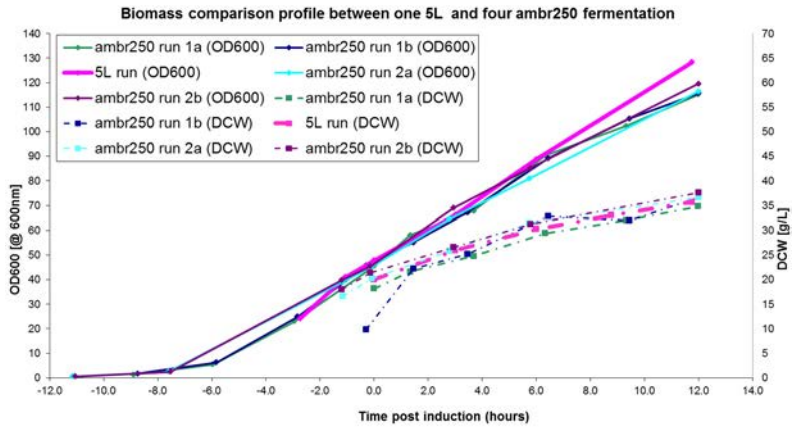


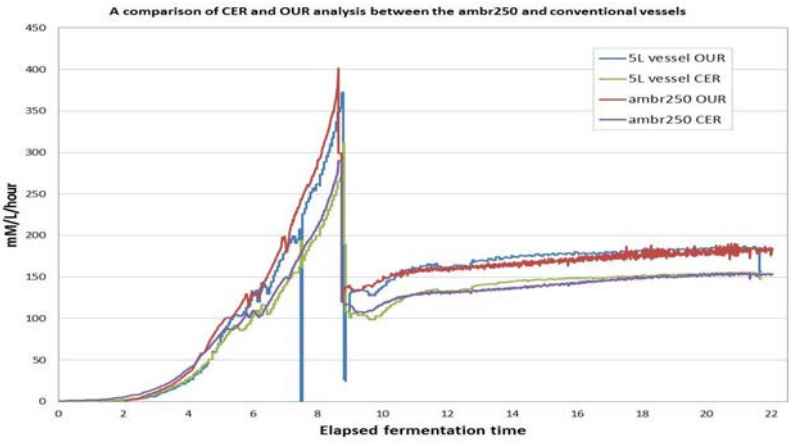
***Figure 2*: Confidence in reproducibility is demonstrated above, where 23 vessels were run using an identical *E. coli* fermentation process (vessel 24 was used as a seed).** *(a) – overlay traces of on-line dissolved oxygen (DO) and agitation (stir speed) for each vessel. (b) – overlay traces of oxygen uptake rate and carbon dioxide evolution rate for each vessel. (c) – overlay traces of off-line biomass measurements via optical density (OD_{600} nm) and dry cell weight (DCW) for each vessel. (d) – HT Caliper analysis of product titres shows reproducibility between the vessels.*

The scalability of the system was confirmed when comparing the results from identical processes run in the ambr250 and conventional 5L bioreactors (*Figure 3*). Comparable results were obtained at the two different scales, e.g. for on-line gas analysis, as well as off-line monitoring of growth, titre and biomass. In fact, the system has been shown to run processes scalable from 5L up to 3000L (data not shown). This ensures that any results obtained are applicable to large scale manufacturing equipment and facilities, and provides confidence that relevant decisions can be made.

A



B



C

	Titre (g/L)
5L	9.6
ambr 250 1a	8.7
ambr 250 1b	9.5
ambr 250 2a	10.3
ambr 250 2b	10.5

***Figure 3*: Demonstrating confidence in scalability** *(A) – Comparable biomass growth profiles. (B) – Comparable CER and OUR traces and (C) Comparable product titres were obtained when running platform FUJIFILM Diosynth Biotechnologies' Escherichia coli fermentation processes using the both ambr250 system and conventional bioreactors.*

COMBINING HIGH THROUGHPUT AND PLATFORM TECHNOLOGY FOR PROCESS OPTIMISATION – EXAMPLE 1

An example of how the ambr250 and high throughput analysis tools have been combined successfully with platform fermentation technology for process optimisation is shown in *Figure 4*. Using Fujifilm's pAVEway expression system and platform fermentation process for *E. coli* intracellular expression, a DoE approach was applied to investigate various fermentation operating parameters with the aim of testing robustness and identifying operating ranges for optimal product titre. The process used a glycerol carbon source with a linear feed initiated following depletion and induction with IPTG once a suitable biomass had been reached. Following a 12 hour induction period the cultures were harvested and product titre determined by Caliper analysis. Twelve fermentations were carried out (including one seed) and the operating parameters investigated were temperature, feed rate, dissolved oxygen concentration and pH. Low, medium and high values assigned for each and a DoE design created using Design Expert. As can be seen from *Figure 4a*, excellent reproducibility was observed between all three centre point fermentations for on line measurements of CER, as well as off line measurements of titre and biomass analysis. Off line measurements of product titre via Caliper analysis and SDS-PAGE were also taken (*Figure*

4b), with statistical modelling revealing that the process was not robust with regards to temperature, dissolved oxygen and feed rate ($p \leq 0.05$). The prediction profiler obtained from the model revealed regions for optimal product titre (*Figure 4c*). Further, the design space generated from the model helped in identifying parameter operating ranges to assure >10 g/L titre production with these ranges adopted for future fermentations (*Figure 4c*).

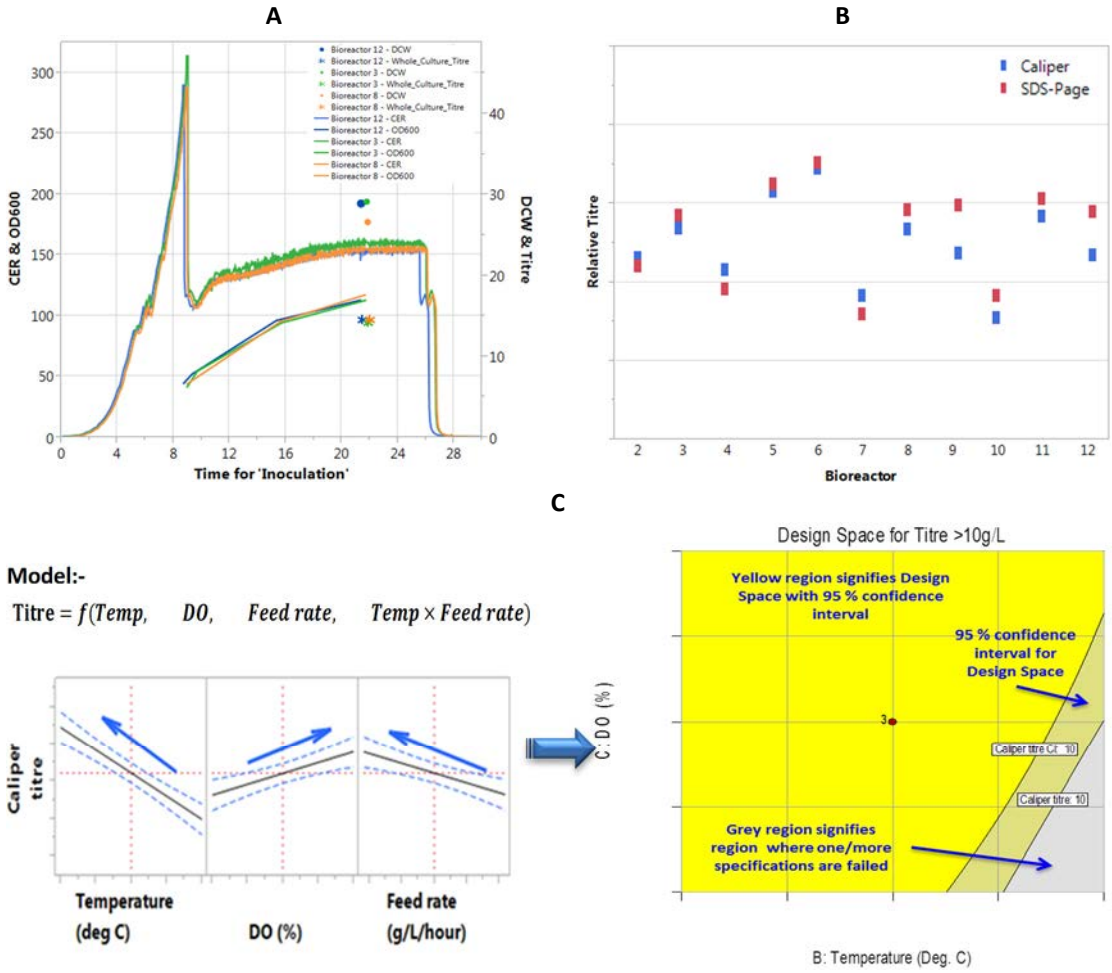


***Figure 4*: A – Centre point reproducibility is demonstrated with overlaying traces of on-line CER and off-line titre, dry cell weight and OD_{600} measurements B – Titre analysis for all DoE fermentations (note, excellent comparability was observed between the Caliper and conventional SDS-PAGE) C – Model as a function of parameters varied in DOE lead to identification of optimal region and suitable design space to be adopted for future fermentations.**

COMBINING HIGH THROUGHPUT AND PLATFORM TECHNOLOGY FOR PROCESS OPTIMISATION – EXAMPLE 2

Further evidence demonstrating the advantages of combining the ambr250 with high throughput and platform fermentation technology for upstream process development is detailed in *Figure 5*. Again using Fujifilm's pAVEway expression system and platform fermentation process for *E. coli* intracellular expression, a DoE approach was used to investigate various fermentation parameters. In this instance, the aim was to determine the effects of parameter operating ranges on soluble intracellular product titres. Previous 15L fermentations had produced high product titres of 17.5g/L

in the form of insoluble inclusion bodies. Due to the nature of the protein and difficulties obtaining suitable refold recoveries post solubilisation, it was investigated whether intracellular soluble product could be produced instead. During the DoE experiment, an exponential feeding strategy was adopted post depletion, along with specified induction temperatures and IPTG induction occurring once the desired OUR had been reached. The operating parameters investigated were induction temperature, exponential growth rate, induction OUR and feed rate cap, with low, medium and high values assigned for each and a DoE design created using Design Expert. Again, excellent reproducibility was observed between the three centre point fermentations for on line and off line measurements (*Figure 5a*). CER and OUR analysis showed distinct groupings between the fermentations(*Figure 5b*), and off line OD_{600} biomass and soluble protein titre measurements were also determined at 12 and 16 hours post induction (*Figure 5c and 5d*). Statistical analysis revealed temperature to have a significant impact ($p< 0.05$) on soluble product titres, with the centre point parameters and a 12 hour induction period being optimal for maximum soluble titres. The process was confirmed at 15L scale, with final titres of 16g/L and around 30% soluble protein achieved. This soluble portion was purified without the need for a refold stage and significantly increased final product yields were obtained.

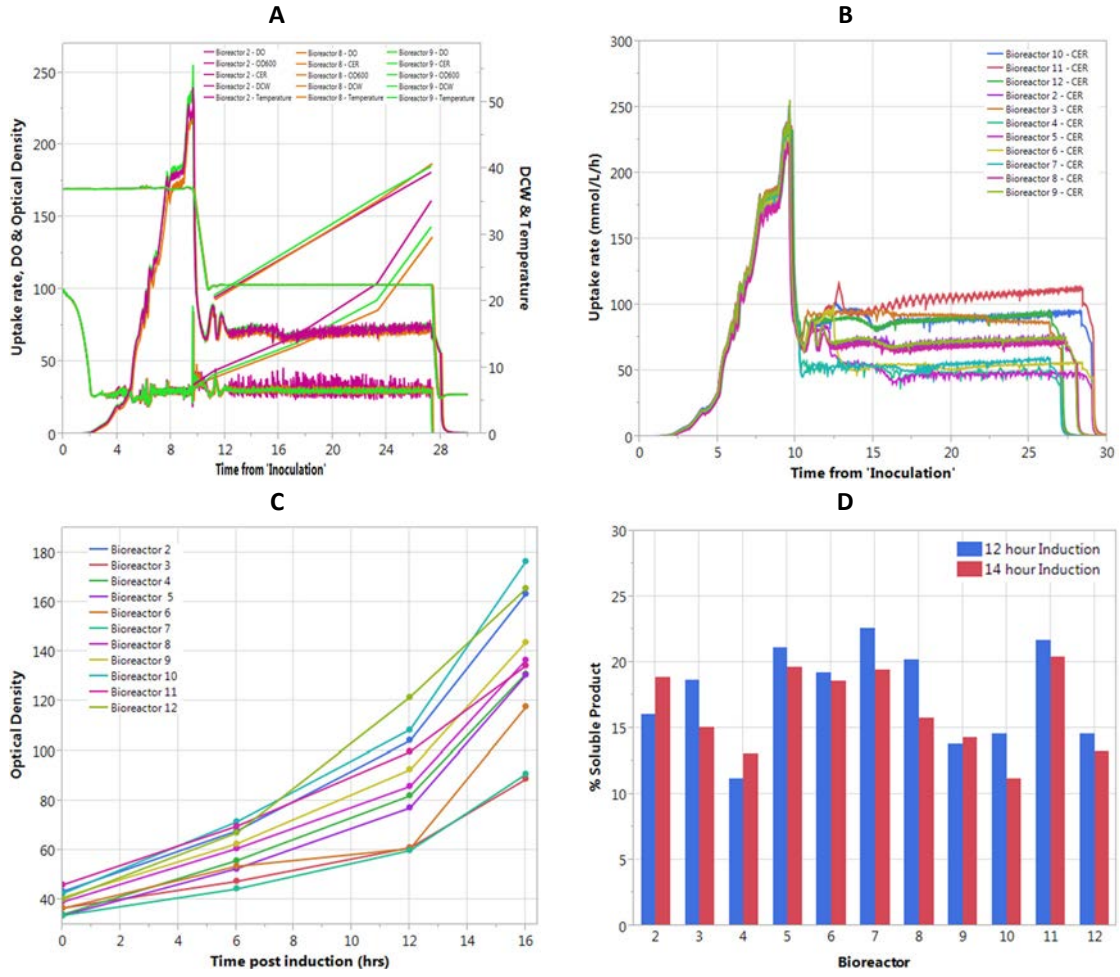


***Figure 5**: A – Centre point reproducibility is demonstrated with overlaying traces of on-line CER and DO, and off-line dry cell weight and OD_{600} measurements B – CER profiles for all DoE fermentations revealing some correlation with post induction feed rate caps D – OD_{600} growth profiles for all DoE fermentations E – Titre analysis for all DoE fermentations, showing around 20% soluble product titres*

CONCLUSIONS

There is a clear need for reproducible, scalable high throughput approaches to upstream development during biotherapeutic production. This is to ensure relevant, high quality data is collected in a manner which reduces waste and ensures confidence when progressing processes for cGMP manufacture. The ambr250 system, as part of a platform development package, helps to meet these needs, with the ability to run 24 parallel fermentations with automated control and continuous monitoring in a system shown by FUJIFILM Diosynth Biotechnologies to run processes scalable up to at least 3000L. Combining this with high throughput analysis tools (for example the Caliper or Cedex BioHT), experimental design and visual analytics software packages (such as JMP and Design Expert) and platform expression systems and fermentation processes (such as the FUJIFILM Diosynth Biotechnologies proprietary pAVEway expression system) enables well characterised processes to be obtained much earlier on in the development process than was possible with previous technology. Late phase characterisation studies can also be performed more rapidly when compared with conventional methods. All this ensures rapid progression from gene to optimised, high titre fermentation process with the in-depth process understanding and robustness required for cGMP manufacture of biotherapeutic drug products.

www.fujifilmdiosynth.com
Email : enquiries@fujifilmdb.com